Original paper

β-Catenin accumulation and S33F mutation of *CTNNB1* gene in colorectal cancer in Saudi Arabia

Suliman Y. Alomar[1], Lamjed Mansour[1], Abdulwahab Abuderman[2], Afrah Alkhuriji[1], Maha Arafah[3], Saleh Alwasel[1], Abdel Halim Harrath[1], Mikhlid Almutairi[1], Paul Trayhyrn[1,4], Javid Ahmad Dar[5]

[1]Zoology Department, King Saud University, Saudi Arabia
[2]Basic Medical Sciences Department, College of Medicine, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia
[3]Department of Pathology, King Saud University, Saudi Arabia
[4]Clore Laboratory, Buckingham Institute for Translational Medicine, University of Buckingham, Buckingham, United Kingdom
[5]Central Laboratory, College of Science, King Saud University, Saudi Arabia

Several risk factors associated with colorectal cancer (CRC) have been identified including *β-catenin/CTNNB1* hotspot mutations. The levels of β-catenin within a cell are regulated via phosphorylation of the *N* terminus of β-catenin by GSK-3β. Thus far three serines (S33, 37, 45) and one threonine (T41) are considered to be the substrates for GSK-3β phosphorylation. In the present investigation an attempt was made to study the role of β-catenin mutations in exon-3 in 60 colorectal cancer patients from Kingdom of Saudi Arabia (KSA). The hot spot mutation region of β-catenin exon 3 was evaluated in matched tumor and normal tissues using PCR and direct sequencing. Sequencing of exon 3 of the *CTNNB1* gene revealed an activating mutation (S33F) in one of the tumor samples as compared to the normal tissue from the same patient where there was no such mutation found. Immunohistochemical staining showed the accumulation of β-catenin protein both in cytoplasm and in the nuclei of cancer cells as compared to normal tissue.

Key words: colorectal cancer, β-catenin, somatic mutation, SNP, PCR-sequencing.

Introduction

The global occurrence of colorectal cancer (CRC) is third among cancer incidence in males and fourth among females [1, 2]. In Saudi Arabia CRC is less common as compared to its counterpart Gulf Cooperation Council States (GCCS) and the West. However, CRC was the second most common malignancy after breast cancer, and first most common malignancy among men between 1994 and 2004 as reported by Saudi Cancer Registry (SCR) [3]. The World Health Organization (WHO) reported in 2004 that the age-standardized death rate from CRC was 8.3% in KSA [4]. The incidence of CRC is increasing among the local population of Saudi Arabia as seen by a three-fold rise in incidence in males from 3.2% to 11.2% within around 7 years; a parallel trend in females was observed with an increase from 2.7% to 8.8% for the same period [3, 5]. Through established surveillance programs and subsequent early detection and removal of pre-cancerous colonic polyps, the incidence of CRC and its related deaths have decreased over the past 15 years in the United States, the high CRC incidence country [6, 7]. In contrast, due to the

lack of surveillance programs and insufficient molecular investigations, the increasing CRC incidence in KSA suggests a potentially alarming situation developing in the coming decades [8].

It has been proposed that chromosomal abnormalities, genetic mutations, and epigenetic changes are the factors responsible for advancement of malignant carcinoma from benign colorectal adenoma [9]. These events lead to inactivation of DNA mismatch repair genes and tumor suppressor genes, or activation of oncogenes. The critical gene mutations in the development of colorectal cancer have been found in adenomatous polyposis coli (APC), Kirstenras (K-ras), p53 and β-catenin genes [10, 11, 12, 13]. Nonetheless, the initiating event for most of the colorectal cancers is observed to be due to the mutations in components of the Wnt/β-catenin pathway [14].

The N-terminus of β-catenin harbors highly conserved residues, S33, S37, S45, and T41 encoded by exon 3 of the human *CTNNB1* gene. These amino acids are not only putative GSK-3 phosphorylation sites but also constitute a part of a 6-amino acid stretch important for ubiquitination, similar to I-kB [15, 16]. Mutation of any one of these amino acid residues in exon 3 of the *CTNNB1* gene generates a stabilized form of β-catenin which can no longer be phosphorylated and degraded and ultimately leading to constitutively active transactivation complexes, which appear to contribute to loss of cell growth control [17, 18]. Different types of tumors have been screened for mutations in exon 3 of the *CTNNB1* gene and, indeed, mutations are found in these four residues [19, 20, 21, 22, 23]. These analyses indicate that a mutation in only one of these phosphorylation sites is sufficient to create a dominant positive form of β-catenin.

The available knowledge about the genetic factors that determine the development and clinical course of colorectal cancers in Eastern and Middle Eastern countries is limited. To date, the association between gene polymorphisms in exon 3 of β-*catenin* gene and the development of colorectal cancer in Saudi population has not yet been investigated. In the present study we tried to identify the mutations in exon 3 of β-*catenin* in primary colorectal carcinomas using PCR-based sequencing analysis.

Experimental methodology

Patient selection and tissue preservation

The study was performed based on approval by the Institutional Review Board of the Ethics Committee at King Khalid University Hospital in Riyadh, KSA. A total of 60 Saudi patients with CRC diagnosed with CRC with available formalin fixed and paraffin embedded tumor and normal tissues were selected from the Department of Pathology and Surgical Oncology

King Khalid Hospital, Riyadh Saudi Arabia. Clinical and demographic features were recorded, including age at the time of diagnosis, gender, nationality, family history, smoking habits, disease behavior, disease location, and type of surgery.

DNA extraction and purification

DNA was extracted from fresh and formalin-fixed and paraffin-embedded (FFPE) tissue specimens by standard techniques as described previously [24]. From all CRC tissue samples 5 micron sections were cut from each FFPE block and mounted onto glass slides. Subsequently, tissue sections were deparaffinized in xylene followed by rehydration in a series of graded ethanol solutions and lastly with ultra-pure water. Using standard protocols one section from each block was stained with haematoxylin and eosin. An expert histopathologist examined and confirmed the presence of tumor tissue in FFPE blocks. Corresponding FFPE tumor tissue blocks were used for DNA extraction using QIAamp DNA FFPE Tissue Kit (Qiagen).

Mutation analysis of exon 3 of *CTNNB1* gene

DNA was extracted from two 20-μm-thick formalin-fixed, paraffin-embedded tissue rolls cut from blocks with at least 70% tumor using the QIAamp DNA mini kit DNA isolation kit (Qiagen Valencia, CA). The genomic DNA isolated from tumor and normal control tissue (n = 60) from the same patient was used as substrate for amplification of exon 3 of the *CTNNB1* gene. The polymerase chain reaction (PCR) used primers *BCAT-F*:5'-ATTTGATGGAGTTGGACATGGC-3' and *BCAT-R*:5'-CCAGCTACTTGTTCTTGAGTGAAGG-3', which yielded an amplicon measuring 224 bp in size with thermocycling conditions previously reported to amplify exon 3 of *CTNNB1* with appropriate controls. PCR products were detected by gel electrophoresis in 2% agarose, and amplicon bands were purified using the QIAquick gel extraction kit (Qiagen). Sequencing of these products were performed with forward and reverse primers by Macrogen Inc. Obtained sequences were analyzed and aligned with CTNNB1 reference sequence from the Gen Bank database accession number, NG_013302.

All PCR and sequencing experiments were repeated at least two times. DNA mutations were confirmed by re-sequencing from an independent PCR reaction using same primers in the same sample.

Immunohistochemistry

Standard immunohistochemistry was performed on formalin fixed, paraffin embedded tissue using an anti-β-catenin monoclonal antibody (Sigma-Aldrich) and performed with ultraView DAB Detection Kit

(Ventana, Arizona, USA) on a BenchMark XT automated staining system (Ventana, Arizona, USA). Tissue sections 3 micron thickness were cut into a coated slides by using Leica RM2235 Rotary Microtome (Leica Biosystems, Wetzlar, Germany), incubated for 15 minutes in a hot air oven at 60°C. The tissue sections were deparaffinized with EZ Prep (Ventana, Arizona, USA) at 75°C, heat pretreated in Cell Conditioning 1 (CC1; Ventana, Arizona, USA) using "standard cell conditioning" for antigen retrieval at 100°C, and then incubated with the anti-β-catenin primary antibody (diluted 1 : 50) for 32 min at 37°C after inactivation of the endogenous peroxidase with hydrogen peroxide for 4 min. Ultraview universal HRP multimer was then added as the secondary

Table I. Demographic and main clinical data of CRC patients used for mutation analysis of β-catenin gene

Demographic details of cancer patients (60)		Number of patients (%)
Gender	male	30 (50)
	female	30 (50)
Average age (years)	male	54.77
	female	57.10
Localization	colon	55 (91.7)
	rectum	5 (8.3)
Cell type	adenocarcinoma	55 (91.7)
	mucinous carcinoma	5 (8.3)
Grade	II	53
	III	5
Stage	T0 N0 M0	1
	T1 N1 Mx	1
	T2 N0 Mx	9
	T2 N1 Mx	1
	T2 Nx Mx	1
	T3 N0 M0	3
	T3NO M1	1
	T3 N0 Mx	19
	T3 N1 Mx	9
	T3 N2 M0	1
	T3 N2 M1	1
	T3 N2 Mx	6
	T4 N0 Mx	3
	T4 N1 M1	1
	T4 N2 Mx	1
	T4 N3 M1	1

antibody. The immunolocalized β-catenin protein was visualized using a copper-enhanced DAB reaction. The slides were counterstained by Hematoxylin II and Bluing Reagent (Ventana, Arizona, USA) for 4 min and then liquid coverslip (LCS) was applied atop aqueous reagents by the NexES Special Stains automated slide stainer to prevent reagent evaporation and ensure complete slide coverage. Following that, samples for mounting in DPX were dehydrated by passing through graded alcohols: 70% ethanol, 96% ethanol and absolute ethanol. Then a small drop of DPX was added to the specimen. After each step Reaction Buffer was used to wash slides to provide a stable aqueous environment. Immunostained sections were reviewed by Olympus BX51 light Microscope and DP72 Olympus Digital Camera (magnification 100× and 200×) (Olympus America Inc, Center Valley, PA, USA).

Results

All the 60 CRC samples were screened for mutations in exon 3 of the β-catenin gene. The demographic and clinic data of the 60 patients enrolled in this study were reported in Table I.

The average age of patients was 55.9 and the number of male and female was the same. The types of tumors are adenocarcinoma for 91.7% located in the colon and 8.3% are mucinous carcinoma located in the rectum. The majority of patients (88.3%) were in grade II of CRC.

Sequencing of exon 3 of the *CTNNB1* gene revealed an activating mutation (S33F) in one of the tumor samples (Fig. 1). The tumor and adjacent normal tissue was obtained from a 63 years old female patient with cecal cancer and retroperitoneal mass. Our data thus indicate for the first time that β-catenin may play a role in the development of colorectal carcinoma and that activating mutations of the β-catenin gene may substitute biallelic *APC* inactivation in this tumor type in KSA.

The mutation at codon 33 changed serine, a hydrophyllic neutral amino acid to phenyalanine, a hydrophobic amino acid {TCT (Ser) → TTT (Phe)}. It is noteworthy that this mutation was heterozygous as evidenced by the presence of double peak pattern in the chromatogram of the tumor DNA in codon 33. Sequencing analysis of the corresponding non-tumorous tissue did not reveal a mutation (Fig. 1).

To determine the β-catenin protein expression level in tumor and the adjacent normal tissue, we performed immunohistochemical analysis on formalin-fixed, paraffin-embedded tissue using anti β-catenin monoclonal antibody. The tumor cells with the S33F mutation displayed an increased protein expression versus that in normal adjacent cells with wild type β-catenin protein (Fig. 2). The enhanced protein
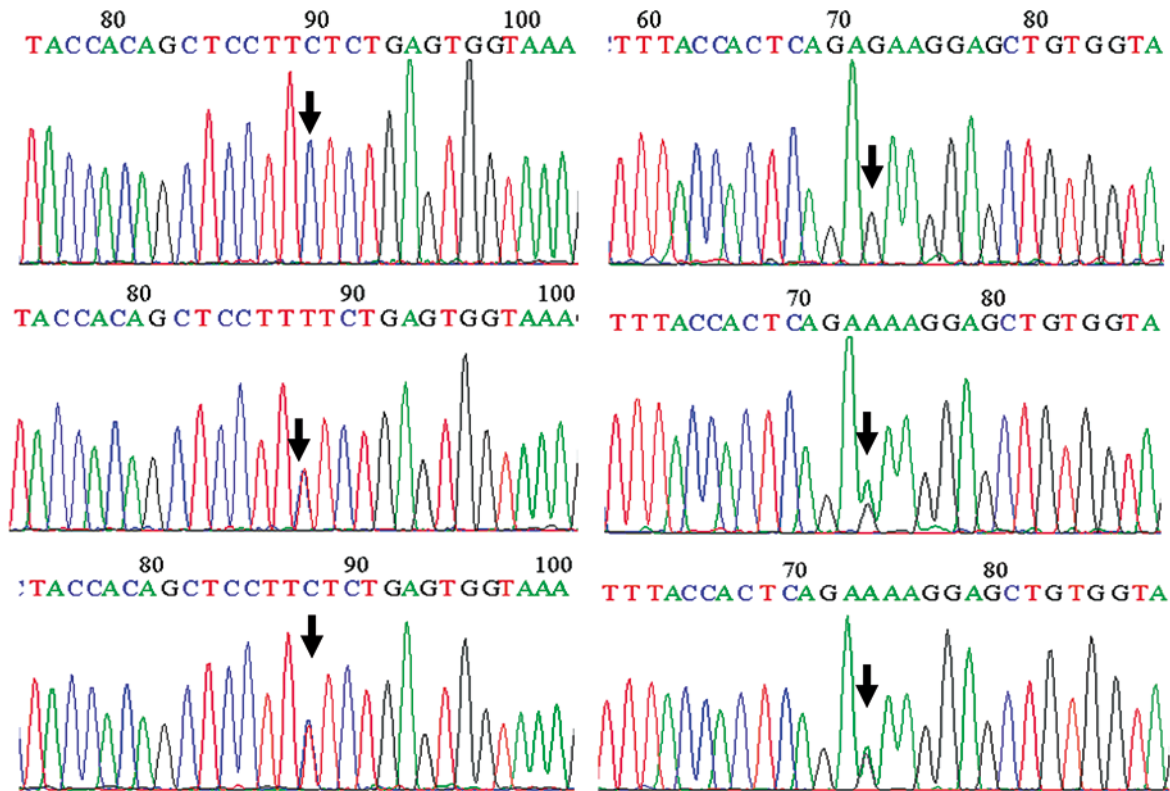
Fig. 1. Sequence analysis of CTNNB1 exon 3 in colorectal cancer and corresponding normal tissue from the same patient. Heterozygous mutation at codon 33 (TCT to TTT) in tumor. Chromatogram of *CTNNB1* exon 3, (A): DNA from normal tissue with wild type codon TCT (S33) sequenced with forward primer and with reverse primer (B), (C): DNA from tumor tissue with heterozygous mutation S33F (TTT > TCT) sequenced with forward primer and with reverse primer (D), (E): DNA from earlier dysplastic lesion with heterozygous mutation S33F (TCT > TTT) sequenced with forward primer and with reverse primer (F)

expression in S33F mutated tumor cells was localized to both cytoplasm and nuclei.

Discussion

β-Catenin plays a key role in the Wnt signaling cascade. β-Catenin is mutated in up to 10% of all sporadic colorectal carcinoma by point mutations or inframe deletions of the serine and threonine residues that are phosphorylated by GSK-3β [14]. These mutations result in stabilization of β-catenin and activation of WNT-signaling. Mutations in β-catenin occur in exclusivity to APC aberrations as both molecules are components of the same pathway [25]. It has been found that the cells containing a mutant APC have elevated β-catenin levels and 80% of colon cancers possess APC mutations [26]. β-Catenin is assumed to transactivate mostly unknown target genes, which may stimulate cell proliferation (acts as an oncogene) or inhibit apoptosis. The β-catenin level in the cell is regulated by its association with the adenomatous polyposis coli (APC) tumor-suppressor protein, axin, and GSK-3β. Phosphorylation of β-catenin by the APC-axin-GSK-3β complex leads to its degradation by the ubiquitin-proteasome system [27, 28, 29]. Thus far three serines (S33, 37, 45) and one threonine

(T41) are considered to be the substrates for GSK-3β phosphorylation. Indeed, these amino acids are regularly mutated in tumors, resulting in accumulation of β-catenin molecules with enhanced transcriptional activity [30].

Our data indicate for the first time in Saudi Arabia that mutations are detected at codon 33 of *β-catenin/ CTNNB1* gene in colorectal cancer tissue. In this study, we have analyzed the status of β-catenin protein and of exon 3 of the *CTNNB1* gene in colorectal cancer for the first time in KSA. Compared to normal adjacent tissue, we found a clear increase of cytoplasmatic and nuclear β-catenin concentration in the malignant cells of one of the 60 investigated cases. Thus, although the number of investigated cases is small and incidence of S33F mutation is very low; our results suggest a possible role of β-catenin accumulation as a result of S33F mutation in the pathogenesis of colorectal cancer in some of the patients in. Our observations need to be confirmed on a larger number of tumors in Saudi Arabia.

An association of β-catenin accumulation and malignancy has been shown for several tumor types, including colorectal carcinomas [21, 31, 32], hepatocellular carcinomas [12, 33], endometroid ovarian carcinomas [34], melanomas [35], and desmoid tu-
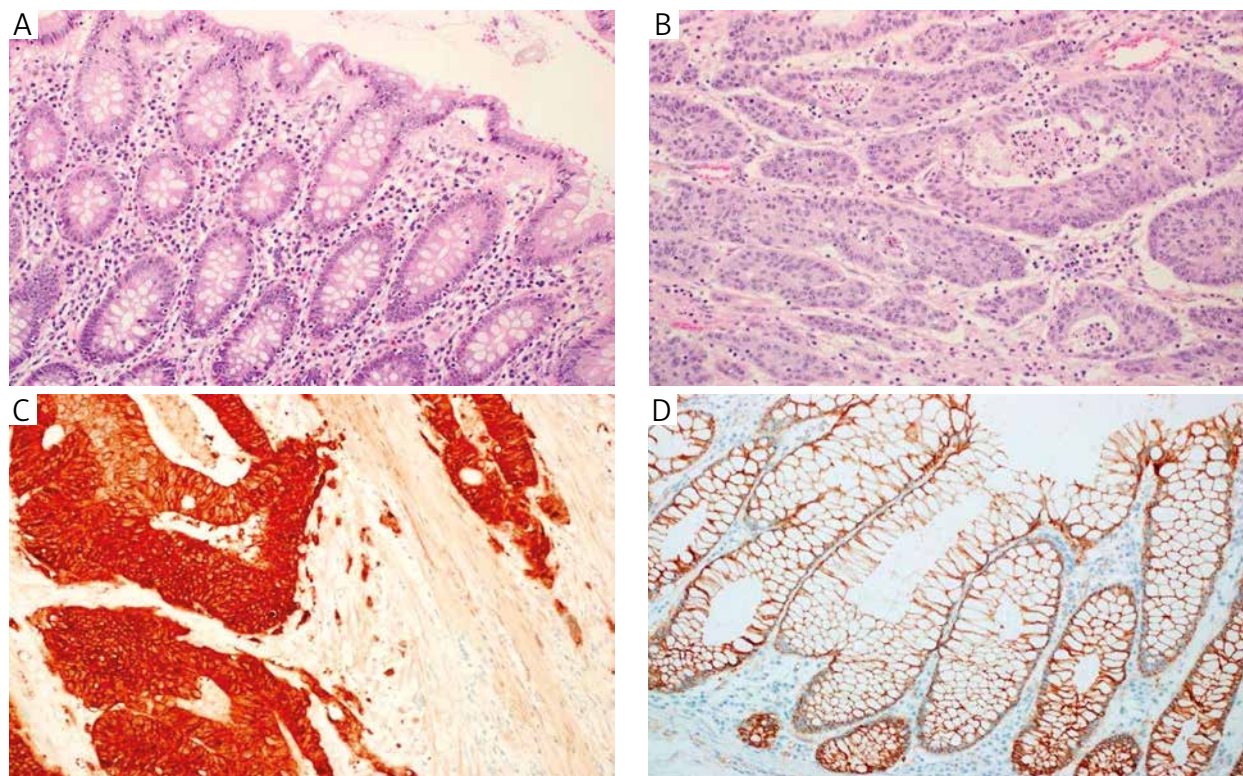
Fig. 2. Protein expression of β-catenin in normal colon and colorectal cancer tissue from the same patient. Normal colon (A) and colorectal cancer (B) stained with H & E. Note strong nuclear and cytoplasmic localization of β-catenin in the tumor cells (C) and weak staining in normal tissue (D). Magnification, A, B, E = 10×; C, D, F = 20×

mors [36, 37, 38]. Although, the increased oncogenic β-catenin activity in these heterogeneous tumor types may be as a result of different mechanisms but colorectal carcinomas, desmoid tumors, and hepatoblastomas have in common that they more frequently affect patients with FAP (familial adenomatous polyposis). However, the most probable mechanism of β-catenin accumulation in FAP-associated tumors is its decreased degradation due to APC dysfunction. The same mechanism is likely to be true for sporadic cases of the above-mentioned tumor types, in which biallelic somatic inactivation of APC have been shown [39, 40, 41, 42]. It has been found that the alterations of the β-catenin itself could also be responsible for elevated protein levels. Mutations in *CTNNB1* gene, which substitute APC inactivation on a molecular level, have recently been described in colorectal carcinoma cell lines [21, 43] as well as in colorectal cancer patients [31]. The mutations were found in exon 3 of the *CTNNB1* gene and resulted in an amino acid change at GSK-3β phosphorylation sites at codons 33, 37, 41, and 45. Lack of phosphorylation at any of these sites decreases sequestration of β-catenin by APC [44]. Our results show for the first time in KSA that *CTNNB1* mutations that may substitute for biallelic *APC* inactivation also occur in colorectal cancer. We found a missense mutation in codon 33 of the *CTNNB1* gene in one of the investigated cases. The mutation (TCT to TTT) results in a substitution of phenylalanine for a serine phosphorylation site. Mutations of the same codon (S33) has earlier been described for hepatocellular carcinoma [45], glomangiopericytoma [46], endometrial hyperplasia [47], brain tumours [48] and colon cancer cells [43] and is considered oncogenic.

In conclusion, our results indicate that colorectal cancer is a tumor in which β-catenin accumulation is implicated in tumor development. As a genetic reason for protein accumulation, we have identified an activating mutation in the *CTNNB1* gene in one of the analyzed cases. Furthermore, the cause of this genetic mutation needs to be investigated with relation to toxicogenetic effects of changed lifestyle and environment. Larger numbers of colorectal cancers should be screened for β-catenin alterations in order to evaluate the validity of our findings.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.

2. Network ICS. Colorectal Cancer: Incidence and Mortality Rates. National Cancer Institute, United States. 2008. http://healthcaredelivery.cancer.gov/icsn/colorectal/mortality.html?&url=/icsn/colorectal/mortality.html&subsite=icsn. Accessed September 20th 2015.

3. Saudi Cancer Registry (SCR) Cancer Incidence and Survival Report Saudi Arabia. Ministry of Health, King Saudi Arabia. 2007. http://www.scr.org.sa/reports/SCR2004.pdf. Accessed June, 10th 2015.

4. Al-Ahwal MS, Shafik YH, Al-Ahwal HM. First national survival data for colorectal cancer among Saudis between 1994 and 2004: what's next? BMC Public Health 2013; 13: 73.

5. Alsanea N, Abduljabbar AS, Alhomoud S, et al. Colorectal cancer in Saudi Arabia: incidence, survival, demographics and implications for national policies. Ann Saudi Med 2015; 35: 196-202.

6. Jackson-Thompson J, Ahmed F, German RR, et al. Descriptive epidemiology of colorectal cancer in the United States, 1998-2001. Cancer 2006; 107: 1103-1111.

7. Rim SH, Seeff L, Ahmed F, et al. Colorectal cancer incidence in the United States, 1999-2004: an updated analysis of data from the National Program of Cancer Registries and the Surveillance, Epidemiology, and End Results Program. Cancer 2009; 115: 1967-1976.

8. Ibrahim EM, Zeeneldin AA, El-Khodary TR, et al. Past, present and future of colorectal cancer in the Kingdom of Saudi Arabia. Saudi J Gastroenterol 2008; 14: 178-182.

9. Bosman F, Yan P. Molecular pathology of colorectal cancer. Pol J Pathol 2014; 65: 257-266.

10. Leslie A, Pratt NR, Gillespie K, et al. Mutations of APC, K-ras, and p53 are associated with specific chromosomal aberrations in colorectal adenocarcinomas. Cancer Res 2003; 63: 4656-4661.

11. Maniatis T. A ubiquitin ligase complex essential for the NF-kappaB, Wnt/Wingless, and Hedgehog signaling pathways. Genes Dev 1999; 13: 505-510.

12. Miyoshi Y, Ando H, Nagase H, et al. Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. Proc Natl Acad Sci U S A 1992; 89: 4452-4456.

13. Ozgüven BY, Karaçetin D, Kabukçuoğlu F, et al. Immunohistochemical study of E-cadherin and beta-catenin expression in colorectal carcinomas. Pol J Pathol 2011; 62: 19-24.

14. Polakis P. Wnt signaling and cancer. Genes Dev 2000; 14: 1837-1851.

15. Beahrs OH. Colorectal cancer staging as a prognostic feature. Cancer 1982; 50: 2615-2617.

16. Bienz M, Clevers H. Linking colorectal cancer to Wnt signaling. Cell 2000; 103: 311-320.

17. Brembeck FH, Rosario M, Birchmeier W. Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. Curr Opin Genet Dev 2006; 16: 51-59.

18. Chun P, Wainberg ZA. Adjuvant Chemotherapy for Stage II Colon Cancer: The Role of Molecular Markers in Choosing Therapy. Gastrointest Cancer Res 2009; 3: 191-196.

19. Blum CA, Tanaka T, Zhong X, et al. Mutational analysis of Ctnnb1 and Apc in tumors from rats given 1,2-dimethylhydrazine or 2-amino-3-methylimidazo[4,5-f]quinoline: mutational 'hotspots' and the relative expression of beta-catenin and c-jun. Mol Carcinog 2003; 36: 195-203.

20. Ilyas M, Tomlinson IP, Rowan A, et al. Beta-catenin mutations in cell lines established from human colorectal cancers. Proc Natl Acad Sci U S A 1997; 94: 10330-10334.

21. Morin PJ, Sparks AB, Korinek V, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997; 275: 1787-1790.

22. Sparks AB, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. Cancer Res 1998; 58: 1130-1134.

23. Johnson V, Volikos E, Halford SE, et al. Exon 3 beta-catenin mutations are specifically associated with colorectal carcinomas in hereditary non-polyposis colorectal cancer syndrome. Gut 2005; 54: 264-267.

24. Soong R, Iacopetta BJ. A rapid and nonisotopic method for the screening and sequencing of p53 gene mutations in formalin-fixed, paraffin-embedded tumors. Mod Pathol 1997; 10: 252-258.

25. Behrens J. The role of the Wnt signalling pathway in colorectal tumorigenesis. Biochem Soc Trans 2005; 33: 672-675.

26. Fearnhead NS, Britton MP, Bodmer WF. The ABC of APC. Hum Mol Genet 2001; 10: 721-733.

27. Amit S, Hatzubai A, Birman Y, et al. Axin-mediated CKI phosphorylation of β-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev 2002; 16: 1066-1076.

28. Zhao J, Yue W, Zhu MJ, Sreejayan N, Du M. AMP-activated protein kinase (AMPK) cross-talks with canonical Wnt signaling via phosphorylation of beta-catenin at Ser 552. Biochem Biophys Res Commun 2010; 395: 146-151.

29. Hino S, Tanji C, Nakayama KI, Kikuchi A. Phosphorylation of beta-catenin by cyclic AMP-dependent protein kinase stabilizes beta-catenin through inhibition of its ubiquitination. Mol Cell Biol 2005; 25: 9063-9072.

30. Verheyen EM, Gottardi CJ. Regulation of Wnt/beta-catenin signaling by protein kinases. Dev Dyn 2010; 239: 34-44.

31. Abdelmaksoud-Damak R, Miladi-Abdennadher I, Triki M, et al. Expression and mutation pattern of beta-catenin and adenomatous polyposis coli in colorectal cancer patients. Arch Med Res 2015; 46: 54-62.

32. Akiyama Y, Nagasaki H, Yagi KO, et al. Beta-catenin and adenomatous polyposis coli (APC) mutations in adenomas from hereditary non-polyposis colorectal cancer patients. Cancer Lett 2000; 157: 185-191.

33. Chen J, Liu J, Jin R, et al. Cytoplasmic and/or nuclear expression of beta-catenin correlate with poor prognosis and unfavorable clinicopathological factors in hepatocellular carcinoma: a meta-analysis. PLoS One 2014; 9: e111885.

34. Palacios J, Gamallo C. Mutations in the beta-catenin gene (CTNNB1) in endometrioid ovarian carcinomas. Cancer Res 1998; 58: 1344-1347.

35. Rubinfeld B, Robbins P, El-Gamil M, et al. Stabilization of beta-catenin by genetic defects in melanoma cell lines. Science 1997; 275: 1790-1792.

36. Alman BA, Li C, Pajerski ME, et al. Increased beta-catenin protein and somatic APC mutations in sporadic aggressive fibromatoses (desmoid tumors). Am J Pathol 1997; 151: 329-334.

37. Li C, Bapat B, Alman BA. Adenomatous polyposis coli gene mutation alters proliferation through its beta-catenin-regulatory function in aggressive fibromatosis (desmoid tumor). Am J Pathol 1998; 153: 709-714.

38. Le Guellec S, Soubeyran I, Rochaix P, et al. CTNNB1 mutation analysis is a useful tool for the diagnosis of desmoid tumors: a study of 260 desmoid tumors and 191 potential morphologic mimics. Mod Pathol 2012; 25: 1551-1558.

39. Hu PJ, Knoepp SM, Wu R, Cho KR. Ovarian steroid cell tumor with biallelic adenomatous polyposis coli inactivation in a patient with familial adenomatous polyposis. Genes Chromosomes Cancer 2012; 51: 283-289.

40. Kurahashi H, Takami K, Oue T, et al. Biallelic inactivation of the APC gene in hepatoblastoma. Cancer Res 1995; 55: 5007-5011.

41. Blaker H, Scholten M, Sutter C, Otto HF, Penzel R. Somatic mutations in familial adenomatous polyps. Nuclear translocation of beta-catenin requires more than biallelic APC inactivation. Am J Clin Pathol 2003; 120: 418-423.

42. Segditsas S, Tomlinson I. Colorectal cancer and genetic alterations in the Wnt pathway. Oncogene 2006; 25: 7531-7537.

43. Wang Z, Vogelstein B, Kinzler KW. Phosphorylation of β-Catenin at S33, S37, or T41 Can Occur in the Absence of Phosphorylation at T45 in Colon Cancer Cells. Cancer Res 2003; 63: 5234-5235.

44. Munemitsu S, Albert I, Souza B, Rubinfeld B, Polakis P. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc Natl Acad Sci U S A 1995; 92: 3046-3050.

45. Miyoshi Y, Iwao K, Nawa G, et al. Frequent mutations in the beta-catenin gene in desmoid tumors from patients without familial adenomatous polyposis. Oncol Res 1998; 10: 591-594.

46. Lasota J, Felisiak-Golabek A, Aly FZ, et al. Nuclear expression and gain-of-function beta-catenin mutation in glomangiopericytoma (sinonasal-type hemangiopericytoma): insight into pathogenesis and a diagnostic marker. Mod Pathol 2015; 28: 715-720.

47. Brachtel EF, Sanchez-Estevez C, Moreno-Bueno G, Prat J, Palacios J, Oliva E. Distinct molecular alterations in complex endometrial hyperplasia (CEH) with and without immature squamous metaplasia (squamous morules). Am J Surg Pathol 2005; 29: 1322-1329.

48. Lee CI, Hsu MY, Chou CH, et al. CTNNB1 (beta-catenin) mutation is rare in brain tumours but involved as a sporadic event in a brain metastasis. Acta Neurochir (Wien) 2009; 151: 1107-1111

Address for correspondence

Lamjed Mansour
Department of Zoology, College of Science
King Saud University
P.O. Box: 2455, Riyadh, 11451, Saudi Arabia
tel. +966550145746
fax +966 11 4678514
e-mail: lamjed.mansour@gmail.com